

GCGACTGGACATCACGAG 3'). Following addition of buffer (30 mM Tris-HCl, pH 7.5, 50 mM NaCl, 60 mM MgCl₂), 120 pmol of substrate (S28A-biotin, 5' biotin-AAAAAAAAAAAAAAAAAAAAAugcacu 3', (SEQ ID NO:34) ribonucleotides in lowercase) was added. The reaction mixture was scaled to accommodate multiple aliquots for each corresponding well of the array. After aliquotting 50 µl into each well of an 96-well PCR plate (MJ Research), 50 µl of ligand in buffer was added. Ligand concentrations for Fig. 29 were: 1 µM 18.90A, 0.5 mM flavin mononucleotide (FMN), 5 µM lysozyme, 1 µM Rev peptide, 1 mM ATP, and 1 mM theophylline.

IN THE DRAWINGS

Figure 19 has been amended and is respectfully submitted herewith. The proposed changes are marked in red ink for approval by the Examiner. Formal drawings will be submitted upon issuance of a Notice of Allowance.

REMARKS

In the Specification, please delete paragraphs [00212] through [00226] and paragraph [00248] and insert the replacement paragraphs as referenced above. A marked-up version of the replacement paragraphs is also attached in accordance with C.F.R. § 1.821. In addition, Applicant respectfully submits the attached drawing with the changes shown in red ink.

This Amendment does not increase the number of claims. If, however, our calculations are in error and a fee is due, please charge this fee to Deposit Account No. 07-0153. Please feel free to telephone the undersigned at the phone number listed below if there are any questions or suggested amendments to the claims.

Dated this April 24, 2002.

Respectfully submitted,
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Serial Number: 09/883,119

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Group Art Unit: 1653

Examiner: N/A

Application Title: REGULATABLE, CATALYTICALLY ACTIVE NUCLEIC ACIDS

MARKED-UP COPY OF AMENDED PARAGRAPHS FOR
PRELIMINARY AMENDMENT IN ACCORDANCE WITH C.F.R. § 1.121

[AMENDED] [00212] Gp1Wt3.129 (TAA TCT TAC CCC GGA ATT ATA TCC AGC TGC ATG TCA CCA TGC AGA GCA GAC TAT ATC TCC AAC TTG TTA AAG CAA GTT GTC TAT CGT TTC GAG TCA CTT GAC CCT ACT CCC CAA AGG GAT AGT CGT TAG) [(SEQ ID NO:17)]. These oligonucleotides (100 pmol) were annealed and extended with AMV reverse transcriptase (Amersham Pharmacia Biotech, Piscataway, NJ; 45 units) in AMV RT buffer (50 mM Tris-HCl, pH 8.3, 8 mM MgCl₂, 50 mM NaCl, 1 mM DTT) and dNTPs (200 µM) for 30 minutes at 37° C. The resulting double-stranded DNA was diluted 1:50 and amplified using primers SpeI.24 (TTA TAC TAG TAA TCT ATC TAA ACG (SEQ ID NO:[18]17); 0.4 µM) and EcoRI.24 (CCC GGA ATT CTA TCC AGC TGC ATG (SEQ ID NO:[19]18); 0.4 µM) in PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 0.1% Triton X-100, 0.005% gelatin), dNTPs (200 µM) and Taq DNA polymerase (Promega, Madison, WI; 1.5 units). The reactions were thermocycled 15 times at 94° C for 30 seconds, 45° C for 30 seconds, 72° C for 1 minute and then purified with a QIAquick PCR purification kit (Qiagen, Valencia, CA).

[AMENDED] [00213] The PCR product was digested with SpeI (New England Biolabs, Beverly, MA; 20 units) and EcoRI (50 units) in buffer (50 mM NaCl, 100 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 0.025% Triton X-100, 100 µg/ml BSA) at 37° C for 60 minutes, purified, and cloned into SpeI/EcoRI digested pTZtd1304. The negative control and nucleic acid constructs were made as described except that Gp1Wt3.129 was replaced with oligonucleotides of the appropriate sequence: B11 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA TCT ATC TAA ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT GCT AAA TGC CTA ACG ACT ATC CCT T (SEQ ID NO:[20]19),

[AMENDED] [00214] Th1P6 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA TCT ATC TAA ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT GCT AAA TTA TAC CAG CAT CGT CTT GAT GCC CTT GGC AGA TAA ATG CCT AAC GAC TAT CCC TT (SEQ ID NO:[21]20),

[AMENDED] [00215] Th2P6 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA TCT ATC TAA ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT GCT AAA TTG ATA

CCA GCA TCG TCT TGA TGC CCT TGG CAG CAT AAA TGC CTA ACG ACT ATC CCT
T (SEQ ID NO:[22]21),

[AMENDED] [00216] Th3P6 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA
TCT ATC TAA ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT GCA TAC CAG CAT
CGT CTT GAT GCC CTT GGC AGG CCT AAC GAC TAT CCC TT (SEQ ID NO:[23]22),--

[AMENDED] [00217] Th4P6 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA
TCT ATC TAA ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT GCT AAA TAT ACC
AGC ATC GTC TTG ATG CCC TTG GCA GTA AAT GCC TAA CGA CTA TCC CTT (SEQ
ID NO:[24]23),

[AMENDED] [00218] Th5P6 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA
TCT ATC TAA ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT ATA CCA GCA TCG
TCT TGA TGC CCT TGG CAG CTA ACG ACT ATC CCT T (SEQ ID NO:[26]24),

[AMENDED] [00219] Th6P6 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA
TCT ATC TAA ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT GAT ACC AGC ATC
GTC TTG ATG CCC TTG GCA GCC TAA CGA CTA TCC CTT (SEQ ID NO:[27]25),

[AMENDED] [00220] Th1P5 TGA GTA TAA GGT GAC TTA TAC TAG TAA TCT
ATC TAA ACG GGG AAC CTC TAT ACC AGC ATC GTC TTG ATG CCC TTG GCA GAG
ACA ATC CCG TGC TAA ATT GTA GGA CTG CCC GGG TTC TAC ATA AAT GCC TAA
CGA CTA TCC CTT (SEQ ID NO:[28]26),

[AMENDED] [00221] Th2P5 TGA GTA TAA GGT GAC TTA TAC TAG TAA TCT
ATC TAA ACG GGG AAC CTA TAC CAG CAT CGT CTT GAT GCC CTT GGC AGA CAA
TCC CGT GCT AAA TTG TAG GAC TGC CCG GGT TCT ACA TAA ATG CCT AAC GAC
TAT CCC TT (SEQ ID NO:[29]27),

[AMENDED] [00222] 3Mex2P6 GTA ATC TAT CTA AAC GGG GAA CCT CTC TAG
TAG ACA ATC CCG TGC TAA ATT GAT ACC AGC ATC GTC TTG ATG CCA TTG GCA
GCA TAA ATG CCT AAC GAC TAT CCC TT (SEQ ID NO:[30]28),

[AMENDED] [00223] Th2P6.D GTA ATC TAT CTA AAC GGG GAA CCT CTC TAG
TAG ACA ATC CCG TGC TAA ATT GAT ACC AGC ATC GTC TTG ATG CCC TTG GTT
GCA TAA ATG CCT AAC GAC TAT CCC TT (SEQ ID NO:[31]29),

[AMENDED] [00224] FMN1P6 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA
TCT ATC TAA ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT GCT AAA TTA GGA
TAT GCT TCG GCA GAA GGA TAA ATG CCT AAC GAC TAT CCC TT (SEQ ID
NO:[32]30), and

[AMENDED] [00225] FMN2P6 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA
TCT ATC TAA ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT GCT AAA TTG AGG
ATA TGC TTC GGC AGA AGG CAT AAA TGC CTA ACG ACT ATC CCT T (SEQ ID
NO:[33]31).

[AMENDED] [00226] In vitro transcription. The introns were PCR-amplified with 5' le (GAT AAT ACG ACT CAC TAT AAT GGC ATT ACC GCC TTG T) (SEQ ID NO:[34]32) and GM24 (GCT CTA GAC TTA GCT ACA ATA TGA AC) (SEQ ID NO:[35]33) in 25 µl reactions under the conditions stated above and cycled 20 times. A portion of the reaction (5 µl) was run on a 3% agarose gel and the PCR product band was stabbed with a pipette tip. The agarose plug was added to a fresh PCR reaction (100 µl) and cycled 15 times; DNA was purified using a QIAquick kit and quantitated. The PCR product (2 µg in 50 µl) was added to an *in vitro* transcription reaction containing Ampliscribe T7 RNA polymerase (Epicentre), RNase inhibitor (GIBCO BRL, Rockville, MD; 5 units), low Mg²⁺ buffer (30 mM Tris-HCl, pH 8, 7.5 mM DTT, 4.5 mM MgCl₂, 1.5 mM spermidine), UTP (1.25 mM), ATP (2.5 mM), GTP (2.5 mM), CTP (7.5 mM) and aP32-labeled UTP (NEN, Boston, MA; 20 µCi; 3000 mCi/mmol), and incubated at 37° C for 2 hours. DNase (GIBCO BRL, 295 units) was added and the reaction was incubated at 37° C for an additional 30 minutes. The RNA was purified using Centri-Sep columns (Princeton Separations, Adelphia, NJ) and quantitated.

[AMENDED] [00248] Aptazyme Array and Titration of Individual Aptazymes. Arrayed aptazyme assay were carried out by first annealing 100 pmol of ribozyme with 120 pmol of 18.90A (5' GCGACTGGACATCACGAG 3')[(SEQ ID NO:36]. Following addition of buffer (30 mM Tris-HCl, pH 7.5, 50 mM NaCl, 60 mM MgCl₂), 120 pmol of substrate (S28A-biotin, 5' biotin-AAAAAAAAAAAAAAAAAAAAAAAAAugcacu 3', (SEQ ID NO:[37]34) ribonucleotides in lowercase) was added. The reaction mixture was scaled to accommodate multiple aliquots for each corresponding well of the array. After aliquotting 50 µl into each well of an 96-well PCR plate (MJ Research), 50 µl of ligand in buffer was added. Ligand concentrations for Fig. 29 were: 1 µM 18.90A, 0.5 mM flavin mononucleotide (FMN), 5 µM lysozyme, 1 µM Rev peptide, 1 mM ATP, and 1 mM theophylline.